

**EFFECT OF HIGH PRESSURE PROCESSING ON WHEAT DOUGH AND
BREAD CHARACTERISTICS**

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21 **ABSTRACT**

22 Microbial, physical and structural changes in high pressured wheat dough were studied
23 as a function of pressure level (50-250 MPa) and holding time (1-4 min). Thereafter,
24 selected conditions of high hydrostatic processing (HPP) were applied to bread dough
25 and the technological quality of the obtained breads was studied. The effect of HPP on
26 wheat dough was investigated by determining microbial population (total aerobic
27 mesophilic bacteria, moulds and yeasts), color and mechanical and texture surface
28 related dough parameters (cohesiveness, adhesiveness, hardness and stickiness). HPP
29 reduced the endogenous microbial population of wheat dough from 10^4 colony forming
30 units/g (CFU) to levels of 10^2 CFU. HPP treatment significantly ($P<0.05$) increased
31 dough hardness and adhesiveness, whereas treatment time reduced its stickiness.
32 Scanning electron micrographs suggested that proteins were affected when subjected to
33 pressure levels higher than 50 MPa, but starch modification required higher pressure
34 levels. HPP treated yeasted doughs led to wheat breads with different appearance and
35 technological characteristics; crumb acquired brownish color and heterogeneous cell gas
36 distribution with increased hardness due to new crumb structure. This study suggests that
37 high hydrostatic processing in the range 50-200 MPa could be an alternative technique
38 for obtaining novel textured cereal based products.

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40

41 **Keywords:** wheat dough, high pressure processing, microbiology, dough texture, bread.

42

43 INTRODUCTION

44 In the last decades, the development of non conventional methods for food processing,
45 like high pressure processing (HPP), has attracted much attention. This technology
46 consists in submitting foods to high hydrostatic pressure (usually among 100 and 1000
47 MPa) with the purpose of inhibiting both pathogen and spoiled microorganisms and of
48 inactivating enzymes that cause undesirable changes (Farr, 1990; Hoover, Metrick,
49 Papineau, Farkas & Knorr, 1989). The application of this technology in food storage has
50 been growing, even at industrial level, due to its effect on microorganisms and enzymes
51 leading to high quality food products (Mertens & Knorr, 1992; Norton & Sun, 2007).
52 Additionally, this technology is being applied to food and raw material processing for
53 obtaining innovative sensorial and functional properties (Welti-Chanes, López-Malo,
54 Palou, Bermúdez, Guerrero-Beltrán & Barbosa-Canovas, 2005; Norton et al., 2007).
55 High hydrostatic pressure has been successfully applied to different food matrices.
56 Currently, it is possible to find fruit juices, oysters, sliced jam, avocado puree, and so on
57 in the market of different countries (San Martín-González, Welti-Chanes & Barbosa-
58 Canovas, 2006; Norton et al., 2007). Nevertheless, there are scarce studies about the use
59 of high hydrostatic pressure in the discipline of cereals and cereal based products. In the
60 Japanese market it can be found HPP treated sake and rice sake (Cheftel, 1995).
61 Different scientific reports described the effect of HPP on specific cereal components
62 properties or model systems, namely starch and gluten (Gomes, Clark & Ledward, 1998;
63 Apichartsrangkoon, Ledward, Bell & Brennan, 1998; Kieffer, Schurer, Köhler & Wieser,
64 2007). HPP induces starch gelatinization, following different mechanism than thermally-
65 induced gelatinization (Gomes et al., 1998). HPP treatment provokes swelling of starch
66 but keeping granule integrity; as a consequence HPP treated starches modify their
67 microstructure and rheological properties in a different way than thermally treated ones

68 (Gomes et al., 1998; Stolt, Oinonen & Autio, 2000), and the extend of swelling highly
69 depends on the type of starch, pressure level and time of treatment (Stute, Heilbronn,
70 Klingler, Boguslawski, Eshtiaghi & Knorr, 1996; Stolt et al., 2000). In addition, thermal
71 properties of pressure-treated starches show decrease in both gelatinization temperature
72 and enthalpy; besides starch granules loose crystallinity and they are prone to aggregate
73 (Wang, Li, Wang, Chiu, Chen & Mao, 2008). Simultaneously, some studies carried out
74 on gluten showed that HPP produces a weakening effect on gluten when low pressure
75 levels (200 MPa) were applied, but an increase in pressure and temperature (800 MPa,
76 60°C) induces the opposite effect, the strengthening of gluten losing its cohesiveness
77 (Kieffer et al., 2007), because HPP brings about the formation of disulphide bonds
78 (Apichartsrangkoon et al., 1998; Kieffer et al., 2007). Nonetheless, there is no study
79 about the effect of HPP on the complete matrix of wheat dough and the potential use of
80 HPP for leading to HPP new cereal based product development with novel texture.

81
82 The aim of this study was firstly to determine the effect of diverse hydrostatic pressure
83 levels applied for different periods of time on the microbiological, physical and
84 structural characteristics of wheat dough and secondly, to explore the possible use of
85 HPP for obtaining wheat breads with novel texture characteristics.

86

87 **MATERIALS AND METHODS**

88 **Materials**

89 A commercial blend of wheat flours (14.21 g/100g moisture content, 11.44 g/100g
90 protein content, 0.61 g/100g ash content, 34.5 g/100g wet gluten) was used in this
91 study. Salt and compressed baker's yeast were acquired in the market.

92

93 **Wheat dough preparation and HPP treatment**

94 Wheat flour was mixed with salt (1.5 g/100g , flour basis), and the amount of water
95 required for reaching 500 Brabender units dough consistency. Mixing was carried out in
96 a 300 g bowl Brabender Farinograph (Brabender, Duisburg, Germany) for 10 minutes.
97 Then dough was divided in 30 g pieces, hand moulded and packed in polyethylene bags
98 (11x12 cm), that were thermosealed under vacuum (Murtivac, España). For HPP, the
99 packed doughs were placed into the pressurization cabinet of the HPP device
100 (Engineering Pressure Systems SO 12644, Belgium), and then subjected to different
101 pressures (50, 100, 150, 200 or 250 MPa) for various holding times (1, 2, 3 or 4
102 minutes). Control dough without HPP treatment was used as reference. Samples after
103 each treatment of pressure and time were evaluated.

104

105 **Microbiological analysis**

106 For determining the endogenous amount of total aerobic mesophilic bacteria (TAMB) in
107 the wheat dough, 10^{-1} and 10^{-2} dilutions of the sample were prepared in water peptone
108 solutions (15g/100 mL), and then 0.1 mL aliquot was inoculated in agar plates (PCA,
109 Scharlau). Colony forming units (CFU) were counted after incubation at 38°C for 24 h..
110 A sample of each dough (1g) was homogenized with 9 mL peptone solutions, serially
111 diluted and plating on potato-dextrose Agar (PDA, Scharlau Chemie, Barcelona, Spain)
112 for yeast and mould counts. Microbial counts were determined after aerobic incubation
113 at 25°C, for five days. Determinations were carried out in triplicate.

114

115 **Dough machinability and surface related profile**

116 Mechanical and surface related properties were determined in the reference (non HPP
117 treated) and in HPP treated doughs. Dough machinability was determined by assessing
118 the texture profile analysis (TPA) and dough stickiness in a TA-XT2i texturometer

119 (Stable Micro Systems, Godalming, UK) as described by [Armero and Collar \(1997\)](#)
120 using the Chen and Hosney cell. Primary textural properties were measured in absence
121 of dough adhesiveness by using a plastic film on the dough surface to avoid the
122 distortion induced by the negative peak of adhesiveness ([Collar & Bollain, 2005](#)). The
123 adhesiveness was measured without the plastic film. Three and ten repetitions for the
124 TPA parameters and stickiness were done, respectively. Compression test was
125 performed with a 25mm diameter cylindrical aluminium probe (SMSP/25), 60%
126 compression rate followed of 75s interval. TPA profile recorded the following
127 parameters: hardness (N), adhesiveness (Ns) and cohesiveness. The Chen and Hosney
128 cell with a cylindrical probe of 25mm diameter was used for dough stickiness (N)
129 determination ([Armero et al., 1997](#)).

130

131 **Wet gluten and gluten index determination**

132 The amount of wet gluten and the gluten index were determined to assess the effect of
133 HPP on the gluten characteristics. Gluten was extracted from 10 g of wheat dough using
134 a gluten washer (Glutomatic, Stockholm, Sweden). Gluten index was determined
135 according to the approved method ([ICC, 2004](#)). Values are the average of three
136 replicates.

137

138 **Scanning electron microscopy**

139 The microstructure of the reference and the treated dough samples was analysed by
140 scanning electron microscopy (SEM). Freeze dried samples were mounted on metal
141 stubs using double tape active carbon and sputter-coated with 100-200Å thick layer of
142 gold and palladium by Ion Sputter (Bio-Rad SC-500). Sample analysis was performed

143 at an accelerating voltage of 10kV with a SEM Hitachi 4100 from the SCSIE
144 Department of the University of Valencia.

145

146 **Breadmaking procedure**

147 Wheat bread doughs or yeasted doughs were obtained following the same procedure as
148 described above for dough preparation but including compressed yeast (4%, flour basis)
149 in the recipe. After HPP treatment, bread dough was hand rounded and put it into pans.
150 Proofing was made in a fermentation cabinet (National MF6.C0, US) at 28°C and 80%
151 relative humidity during the required time to reach three times the initial bread dough
152 volume. Pan breads were baked in an electric oven (Eurofours, France) at 210°C for 11
153 min, and then they were cooled down for one hour before running the further analysis.

154

155 **Dough and bread color**

156 Color was determined with a Color Guard System Colorimeter (Hunter Laboratory,
157 Reston, VA). Color was determined by reflectance mode and expressed by L
158 (luminosity), a (green-red) and b (blue-yellow) Hunter parameters. The colorimeter was
159 calibrated by utilizing the black tile and the white standard ($L=92.89$, $a=-1.05$, $b=0.82$).
160 The net difference of color (ΔE) was calculated by equation 1:

161

$$162 \quad \Delta E = \sqrt{(L_c - L_m)^2 + (b_c - b_m)^2 + (a_c - a_m)^2} \quad (\text{Eq.1})$$

163

164 Where the subscript m corresponded to HPP treated dough or bread obtained from
165 treated bread dough, and subscript c indicated the values of the reference dough or
166 bread (without HPP treatment).

167

168 **Bread quality assessment**

169 In order to determine the bread quality, the volume (rapeseed displacement), weight,
170 height/width ratio of the slices, and moisture content were measured. Moisture content
171 was determined following the [ICC Method \(2004\)](#). Besides, a texture profile analysis
172 (TPA) of the breadcrumbs was performed by a Texture Analyzer TA-XT2i texturometer.
173 A bread slice of 2-cm-thickness was compressed up to 50% of its original height at a
174 crosshead speed of 1 mm/s with a cylindrical stainless steel probe (diameter 25 mm).
175 Values were the mean of four replicates. The cross-section of bread slices was visually
176 observed. Sensory perception was performed by ten trained panellists (5 women and 5
177 men), whose age ranged from 24 to 50 years old. The trained panel scored the overall
178 acceptability of the breads using a semi-structured scale (0: extremely dislike, 10:
179 extremely like).

180

181 **Statistical analysis**

182 Experimental data from wheat dough characterization were submitted to multifactor
183 analysis of variance (MANOVA) using Statgraphics Plus, versión 5.1 (Statistical
184 Graphics Corp., 1994-2001). Experimental data from bread quality assessment were
185 submitted to one-way analysis of variance (ANOVA). All data were presented as mean
186 values of at least three replicates \pm standard error (SE). When analysis of variance
187 indicated significant *F* values, multiple sample comparison was also performed by
188 Tukey HSD test in order to detect significant differences ($P < 0.05$).

189

190 **RESULTS AND DISCUSSION**

191 **Effect of high pressure processing on the microbial population of the wheat dough**

192 A preliminary study of the effect of HPP on wheat dough was carried out to select the
193 most adequate HPP conditions considering the endogenous microbial dough population
194 and dough mechanical and structural properties. The effect of different HPP levels,
195 applied for different duration, on the colony forming units (CFU) of total aerobic
196 mesophilic bacteria (TAMB) of wheat dough can be observed in Figure 1. An important
197 reduction of microorganisms was observed after one minute of exposure at HPP, and no
198 further significant decrease was obtained by increasing the duration of the treatment,
199 with the exception of samples treated at hydrostatic pressure of 50 MPa, which required
200 prolonged treatment (two minutes). Generally, an increase in pressure has been related to
201 high microbial inactivation, but that relationship has not been found with the time of
202 treatment (Palou, López-Malo, Barbosa-Canovas, Welti-Chanes, Davidson & Swanson,
203 1998). In fact, it is necessary to determine the threshold HPP required for each
204 microorganism inactivation and above that an increase in the time of exposition does not
205 promote a significant reduction in the microbial counts, always having in mind the
206 essential role of the environment on the microorganism resistance to HPP (Palou et al.,
207 1998). Nonetheless, HPP below 200 MPa can induce spore germination of certain
208 bacterial strains (Gould & Sale, 1970), increasing the amount of cells in vegetative state
209 and thus the colony forming units, although the present results did not suggest any
210 germination.

211

212 Similarly, it was determined the total moulds and yeasts counts presented in wheat dough
213 treated at different HPP during different time (Figure 1). Regardless the lowest HPP
214 tested, one minute of HPP was enough for reducing the moulds and yeast endogenous
215 population of the wheat dough. Similarly, with the exception of 50 MPa, no further
216 moulds and yeast counts decrease was observed by increasing treatment time. In

217 opposition, when doughs were HPP treated at 50 MPa, moulds and yeasts counts showed
218 additional decrease by extending the treatment time. Moulds and yeast are very sensitive
219 to HPP affecting the external shape of the cells and very high HPP treatment (500 MPa)
220 induces disruption and damage of the cell walls (Ogawa, Fukuhisa, Kubo & Fukumoto,
221 1990; Shimada, Andou, Naito, Yamada, Osumi & Hayashi, 1993).

222 The pairs HPP-time used in this study were enough to promote a significant reduction of
223 the TAMB and moulds and yeast counts, and after one or two minutes of treatment the
224 final microbial populations of the doughs were approximated 10^2 CFU/g. Lately, the
225 effect of HPP on the microorganism has been widely studied showing good efficiency
226 for microorganism inactivation (Carlez, Rosec, Richard, & Cheftel, 1993; Patterson,
227 Quinn, Simpson & Gilmour, 1995; Palou, López-Malo & Welti-Chanes, 2002), but
228 higher pressure and time than the ones used in the present study are usually applied.
229 Very mild HPP level and treatment time were tested in this study to ensure treatment
230 effectiveness with retention of dough functionality.

231

232 **Effect of HPP on wheat dough color**

233 Experimental color data were submitted to statistical analysis to determine the level of
234 significance of HPP level and the treatment duration on the color parameters (L , a , b)
235 and the total color change (ΔE) (Table 1). The color of wheat dough was affected by
236 HPP treatment; nevertheless absolute differences among experimental data were rather
237 small. Luminosity (L) of the samples varied with the HPP treatment and the holding time
238 at constant pressure, but no significant differences were observed between the control
239 (untreated dough) and the treated doughs due to the pressure intensity. Pressure levels of
240 100 and 150 MPa produced the greatest effect on the a and b color parameters, showing
241 a tendency to red and yellow, respectively. Concerning the period of HPP treatment,

242 increasing duration of HPP resulted in decreased values of a parameter, whereas no
243 significant differences of b parameter were observed between the untreated sample (time
244 0) and the HPP treated samples. The total change of color (ΔE) only was significantly
245 ($P < 0.05$) increased when the highest HPP level (250 MPa) was applied, and regarding
246 the duration of HPP, all the times tested affected significantly ($P < 0.05$) this parameter.
247 There is a general assumption that HPP is a preservative technique that protects food
248 color but only once circumspect treatment is applied (Norton et al., 2007). Hydrostatic
249 pressure applied to wheat dough modified the color parameters but only at the highest
250 pressure tested (250 MPa) was observed a significant change of the total color.

251

252 **Effect of HPP on gluten**

253 The amount of wet gluten and gluten index was determined in wheat dough samples
254 subjected to different levels of HPP (Table 2). Only the pressure level applied during
255 HPP induced significant effect on the amount of wet gluten and its quality assessed as
256 gluten index. However, no significant differences were observed between the untreated
257 dough and the HPP treated doughs. The duration of the HPP or holding time at constant
258 pressure did not have any significant effect on the characteristics of the gluten. Some
259 studies carried out on wheat gluten stated that at 20°C, only hydrostatic pressure higher
260 than 200 MPa modifies the gluten structure and the effect was dependent on the holding
261 time (20 or 50 min) and temperature (20-60°C) (Apichartsrangkoon et al., 1998;
262 Apichartsrangkoon, Bell, Ledward & Schofield, 1999). Conversely, Kieffer et al. (2007)
263 observed a decrease in the gluten strength when treated at low pressure (200 MPa).
264 Therefore, slightly contradictory results have been obtained when gluten was treated at
265 low pressure, but a consensus has been reached concerning that changes in gluten
266 structure associated to disulfide cross-linking only became significant at extreme

267 conditions (400-800MPa, 60°C or lower temperature but prolonging the exposure)
268 ([Apichartsrangkoon et al., 1998](#); [Kieffer et al., 2007](#)). HPP conditions applied in the
269 present study did not reveal substantial changes on gluten characteristics, either wheat
270 dough provided a shielding effect on gluten, or HPP conditions were too mild to induce
271 gluten physical changes.

272

273 **Effect of HPP on the texture parameters of wheat dough**

274 Data of mechanical and texture surface related parameters are included in Table 2. The
275 pressure level had a significant effect on the hardness, cohesiveness and adhesiveness,
276 whereas the holding time at constant pressure had only significant effect on the
277 stickiness. Increasing values of hardness and adhesiveness were obtained when raising
278 the hydrostatic pressure, besides a decrease in stickiness when increasing the time of
279 treatment (Table 2). No differences were detected on the cohesiveness of untreated
280 dough and HPP treated wheat doughs. Results agree with [Apichartsrangkoon et al.](#)
281 [\(1998\)](#) findings that described an increase in the hardness of high pressure treated wheat
282 gluten when applying hydrostatic pressure within the range 200-800 MPa, and that effect
283 was markedly dependent on the pressure, temperature and holding time of HPP. It is
284 advisable for proper breadmaking to keep dough stickiness at low levels, because it
285 seriously constrains dough machinability and that is even more important in automated
286 breadmaking processes ([Armero et al., 1997](#)). Considering that dough cohesiveness has
287 been reported as a good predictive parameter of fresh bread quality and keepability, and
288 maximized dough cohesiveness and minimized dough stickiness are recommended
289 trends for providing good bread-making performance ([Armero et al., 1997](#)), the range of
290 HPP conditions applied in this study would provide wheat dough with satisfactory
291 breadmaking performance.

292

293 **Microstructure of HPP treated wheat dough**

294 Scanning electron microscopy was used to determine the effect of the HPP on dough
295 microstructure. Scanning electron micrographs of wheat doughs treated at 50, 150 and
296 250 MPa for four minutes are showed in Figure 2. Untreated wheat dough (0 MPa) was
297 characterized by having a continuous structure with the intact starch granules embedded
298 in the matrix structure of proteins and soluble solutes. Two distinct populations of starch
299 granule sizes could be envisaged, ones with lenticular shape and the others smaller and
300 with spherical shape, which agree with previous reported two populations of A and B-
301 type of starch granules ([Angold, 1975](#)). In the present study the reticular structure of the
302 wheat dough, previously reported ([Rojas, Rosell, Benedito, Pérez-Munuera & Lluch,](#)
303 [2000](#)), was not evident because samples were not subjected to sublimation, which is
304 necessary in cryo-scanning electron microscopy. After HPP treatment the continuous
305 matrix appeared disaggregated and the starch granules were clearly identified as
306 individual structures, however their structure became more distorted as the pressure level
307 increase. Dough treated at pressure of 50 and 150 MPa showed well defined starch
308 granules with diverse size, and the surrounding structures (mainly of protein nature)
309 were progressively reduced, being confined in the case of 150 MPa to agglomerates of
310 starch granules. Drastic changes were observed in dough treated at 250 MPa where
311 starch granules as individual structures disappeared adopting a discontinuous film like
312 organization similar to what happened after swelling and gelatinization. The effect of
313 high hydrostatic pressure on different types of starch has been widely studied ([Stolt et al.,](#)
314 [2000](#); [Stute et al., 1996](#); [Gomes et al., 1998](#); [Katopo, Song & Jane, 2002](#)). High pressure
315 induces a gelatinization process keeping intact the starch morphology ([Stute et al., 1996](#);
316 [Katopo et al., 2002](#)). The effect of HPP on starch granules led to a limited swelling that

317 is highly dependent on the level of pressure, water moisture content, time of exposure,
318 and starch type (Stute et al., 1996). Studies focused on the effect of HPP on diverse
319 proteins described that pressure levels equal or higher than 1000-2000 MPa do not have
320 a significant effect on covalent bonds (Mozhaev, Heremans, Frank, Masson & Balny,
321 1994), hydrogen bonds are induced by HPP treatment and the hydrophobic linkages tend
322 to be stabilized at pressure levels higher than 100 MPa (Hoover et al., 1989). Following
323 previously reported explanations, gluten structure should not be severely affected by the
324 HPP carried out in the present study, but considering that HPP also favors unfolding and
325 dissociation of oligomeric proteins (Masson, 1992), some gluten modification could be
326 expected. Apichartsrangkoon et al. (1998) and Kieffer et al. (2007) found that gluten
327 treatment with HPP (ranged from 200-800 MPa for 20-50 minutes or combining high
328 temperature) yielded the formation of additional disulphide bonds that besides the
329 hydrogen and hydrophobic bonds, formed or altered by the HPP, originated changes in
330 the protein matrix of gluten, which differed from the heat induced ones. Therefore,
331 microscopy studies revealed that HPP treatment up to 150 MPa for four minutes induced
332 microstructural changes on wheat dough related to matrix disorganization, likely due to
333 protein unfolding, but drastic starch modification required higher pressure levels (250
334 MPa).

335

336 **Effect of HPP on technological quality of wheat bread**

337 Studies carried out on wheat dough allowed selecting an intermediate holding time (2
338 min) and hydrostatic pressures ranged from 50-200 MPa for inducing modifications on
339 bread dough (yeasted dough) without loosing its breadmaking performance. Those HPP
340 conditions were applied to bread dough, which were then submitted to the conventional
341 breadmaking process for obtaining wheat breads. Cross-section of the bread slice

342 obtained from HPP treated yeasted doughs is shown in Figure 3. Bread pictures show
343 that HPP greatly affected the crumb microstructure with uneven distribution of the gas
344 cells, and increased size of the alveoli. Even the lowest HPP level (50 MPa) induced the
345 formation of bigger gas cells compared to the control. Large gas cells were observed in
346 some places of the treated crumbs, besides a brownish color, and that appearance was
347 more noticeable when increasing pressure levels. Despite high hydrostatic processing
348 reduced the microbial population in wheat dough, the HPP treated yeasted doughs had
349 enough microbes' survival (around 10^4 CFU/g in all HPP yeasted samples) for ensuring
350 dough fermentations.

351 The sensory evaluation of the treated breads revealed that, with the exception of the
352 200MPa treated sample, HPP gave acceptable products, with the same overall
353 acceptance as the non-treated bread (score 7). Judges emphasized the original layered
354 structure of the treated bread crumb that was very soft and attractive, reminding the
355 croissant structure.

356 Therefore the use of HPP on the yeasted dough might lead to the development of new
357 bakery products with novel crumb characteristics, resembling croissant structure.

358 Regarding bread technological quality (Table 3), HPP significantly ($P<0.05$) reduced the
359 volume and specific volume of the loaves, obtaining more compact and flat breads at
360 higher hydrostatic pressure treatment, as indicated the width/height ratio. Presumably,
361 the effect of HPP either on gluten network structure or on yeast cells fermentation ability
362 led to reduced dough expansion during proofing and baking, and in consequence lower
363 specific volume. Crumb hardness was also modified obtaining higher crumb hardness
364 with breads from treated doughs, which was expected due to the protein network
365 modification induced by HPP. Hardness increase has been always described in breads
366 that have undergone gluten network damage, for instance due to ice crystal formation

367 and growing like in frozen doughs or even from frozen partially baked breads
368 (Bhattacharya, Langstaff & Berzonsky, 2003; Bárcenas & Rosell, 2006).

369 The moisture content of the bread showed a steady increase as function of pressure levels.
370 Likely, crumb structure was holding higher amount of water molecules partly due to
371 chemical pressure-induced changes in proteins (Apichartsrangkoon et al., 1998; Kieffer
372 et al., 2007) and the pressure-induced gelatinization of starch that allows completing
373 starch gelatinization at lower temperatures (Stute et al., 1996; Katopo et al., 2002).

374 Color of crumb and crust of the bread was modified due to the HPP treatment of the
375 bread doughs (Table 4). Luminosity, *a* and *b* color parameters of crust were reduced with
376 the pressure levels, showing a significant ($P < 0.05$) decrease at the higher pressure levels
377 tested. Those results agree with studies carried out by Tamaoka, Itoh and Hayashi (1991),
378 who showed that HPP in the range 50 and 500 MPa at 50° C on model systems formed
379 by glyceraldehyde, glycolaldehyde or xylose and aminoacids did not have any effect on
380 the condensation reaction but suppressed the browning process in the total course of the
381 Maillard reaction. The overall HPP effect considered as the total change of color (ΔE)
382 showed a significant increase.

383 Concerning the crumb color, significant decrease in luminosity together with a large
384 increase in the total change of color were observed, in agreement with the visual
385 observation of the cross-section of the slices (Figure 3). No general trend could be
386 established regarding the *a* and *b* color parameters. Although color changes induced by
387 HPP in wheat dough were rather small, the temperature applied during baking resulted in
388 significant crumb color changes.

389

390

391 Treatment of wheat dough with high hydrostatic pressure induced rapid reduction of the
392 microbial population but sufficient mold and yeast survival, for ensuring bread dough
393 fermentation, can be obtained using mild pressure conditions (50-250 MPa, for two
394 minutes at 20°C). Regarding dough properties, HPP treatment significantly ($P<0.05$)
395 increased hardness and adhesiveness, whereas stickiness was reduced by increasing the
396 time of exposure to HPP. SEM micrographs suggested that proteins were affected at
397 pressure levels ranged from 50 to 150 MPa, but starch modification required higher
398 pressure levels. HPP treated yeasted doughs allowed obtaining wheat breads with
399 different appearance and technological characteristics; crumb acquired brownish color
400 and uneven cell gas distribution with increased hardness due to new crumb structure.
401 This study suggests that high hydrostatic processing in the range 50-200 MPa could be
402 an alternative technique for obtaining novel textured cereal based products.

403

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505 **Table 1.** Effect of high pressure processing on color parameters of
506 wheat dough. Experimental data were submitted to multifactor analysis
507 of variance (MANOVA).
508

	<i>L</i>			<i>a</i>			<i>b</i>			ΔE	
	Mean	SE		Mean	SE		Mean	SE		Mean	SE
Grand mean	80.30			1.88			17.04			3.58	
Pressure level (MPa)											
50	80.64	0.32	ab	1.72	0.15	ab	16.18	0.46	a	3.27	0.23 a
100	79.44	0.39	a	2.54	0.18	c	18.67	0.56	c	3.19	0.29 a
150	79.84	0.39	a	2.07	0.18	bc	18.29	0.56	bc	3.26	0.29 a
200	79.90	0.39	a	1.75	0.18	ab	16.39	0.56	ab	3.55	0.29 a
250	81.70	0.39	b	1.33	0.18	a	15.68	0.56	a	4.64	0.29 b
Treatment time (min)											
0	77.33	0.79	a	2.95	0.37	c	17.55	1.12	ab	1.01	0.23 a
1	81.06	0.32	b	1.74	0.15	b	17.36	0.46	ab	3.98	0.23 b
2	81.26	0.32	b	2.03	0.15	bc	17.98	0.46	b	4.41	0.23 b
3	80.55	0.32	b	1.57	0.15	ab	16.19	0.46	ab	3.99	0.23 b
4	81.31	0.32	b	1.10	0.15	a	16.14	0.46	a	4.53	0.23 b

Means of three replicates followed by different letters within rows and groups were significantly different at $P < 0.05$.

SE: Standard error

509

510 **Table 2.** Effect of high pressure processing on gluten and mechanical and texture surface related parameters of wheat dough. Experimental data
511 were submitted to multifactor analysis of variance (MANOVA).
512

	Wet gluten (g)			Gluten index (%)			Hardness (N)			Cohesiveness			Adhesiveness (Ns)			Stickiness (N)		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Grand mean	16.9			51.1			4.61			0.65			4.34			0.44		
Pressure level (MPa)																		
50	17.3	0.3	ab	53.1	2.9	ab	3.36	0.37	a	0.63	0.02	ab	2.75	0.51	a	0.41	0.03	
100	16.5	0.4	ab	49.0	3.5	ab	3.81	0.45	ab	0.70	0.02	b	2.44	0.62	a	0.41	0.03	
150	16.1	0.4	a	42.3	3.5	a	5.09	0.45	bc	0.65	0.02	ab	6.79	0.62	b	0.46	0.03	
200	17.0	0.4	ab	56.4	3.5	b	5.48	0.45	c	0.67	0.02	ab	5.09	0.62	b	0.46	0.03	
250	17.6	0.4	b	54.9	3.5	ab	5.43	0.45	c	0.60	0.02	a	4.95	0.62	b	0.43	0.03	
Treatment time (min)																		
0	15.9	0.7		50.5	7.0		4.06	0.90		0.63	0.05		4.95	0.52		0.60	0.07	b
1	17.5	0.3		50.6	2.9		4.50	0.37		0.65	0.02		4.70	0.51		0.52	0.03	b
2	16.8	0.3		51.2	2.9		5.63	0.37		0.65	0.02		4.20	0.51		0.36	0.03	a
3	16.7	0.3		48.9	2.9		4.26	0.37		0.68	0.02		3.56	0.51		0.40	0.03	a
4	17.8	0.3		54.4	2.9		4.62	0.37		0.64	0.02		4.30	0.51		0.41	0.03	a

Means of three replicates (ten in the case of stickiness) followed by different letters within rows and groups were significantly different at $P < 0.05$.

SE: Standard error

Values were not followed by letters, when ANOVA indicated no significant F values

Table 3. Effect of different levels of high pressure processing and holding time of two minutes on fresh bread quality parameters. Experimental data were submitted to one-way analysis of variance (ANOVA).

Pressure level (MPa)	Moisture content (g/100g)			Volume (cm ₃)		Specific volume (cm ₃ /g)		Width/Height ratio		Hardness (N)					
	Mean	SE		Mean	SE	Mean	SE	Mean	SE	Mean	SE				
0	29.6	0.0	a	126	7	d	4.2	0.0	c	1.3	0.0	a	2.48	0.10	a
50	30.2	0.0	b	84	2	c	2.8	0.0	b	1.3	0.0	a	5.39	0.10	b
100	34.9	0.0	c	80	1	b	2.7	0.0	b	1.6	0.0	b	6.26	0.10	c
200	35.0	0.0	d	59	2	a	2.0	0.0	a	1.5	0.0	b	15.87	0.10	d

Means of three replicates followed by different letters within rows were significantly different at P<0.05.

SE: Standard error

518 **Table 4.** Effect of different levels of high pressure processing and holding time of two minutes on crust and crumb color parameter of fresh bread.

519 Experimental data were submitted to one-way analysis of variance (ANOVA).

Pressure level (MPa)	Crust												Crumb											
	<i>L</i>			<i>a</i>			<i>B</i>			ΔE			<i>L</i>			<i>a</i>			<i>b</i>			ΔE		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
0	49.87	0.33	b	10.03	0.20	bc	21.54	0.27	b	0.38	0.28	a	60.58	0.99	d	-0.72	0.02	bc	8.61	0.11	b	2.08	0.65	a
50	52.03	0.33	c	9.59	0.20	b	22.76	0.27	b	2.54	0.28	b	53.66	0.99	c	-0.94	0.02	a	10.59	0.11	c	7.22	0.65	b
100	47.24	0.33	a	10.50	0.20	c	17.79	0.27	a	4.69	0.28	c	44.96	0.99	b	-0.65	0.02	c	7.42	0.11	a	15.67	0.65	c
200	45.96	0.33	a	8.48	0.20	a	18.46	0.27	a	5.23	0.28	c	39.62	0.99	a	-0.78	0.02	b	7.38	0.11	a	21.02	0.65	d

Means of three replicates followed by different letters within rows were significantly different at $P < 0.05$.

SE: Standard error

520

FIGURE CAPTIONS

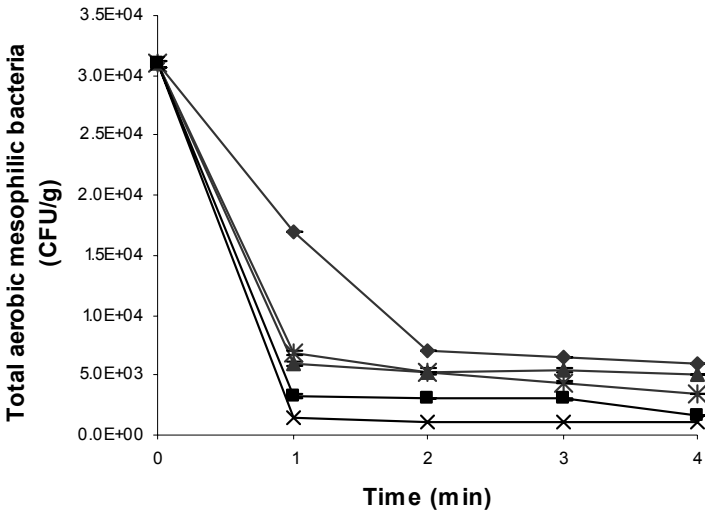
Figure 1. Effect of HPP treatment on total aerobic mesophilic bacteria (A) and on molds and yeasts (B) of wheat dough. Error bars indicate standard deviation (n=3). Symbols: ♦: 50MPa; ▲: 100MPa; ✱: 150MPa; ■: 200MPa; ×: 250MPa.

Figure 2. Scanning electron micrographs (SEM) of wheat dough (2500X) exposed to different levels of high pressure processing (0, 50, 150, 250 MPa) for 4 minutes.

Figure 3. Cross section of the different bread slices obtained from bread doughs treated at different high pressure processing levels and two minutes of exposure. Values indicate the pressure level used for HPP.

Figure 1

A.



B

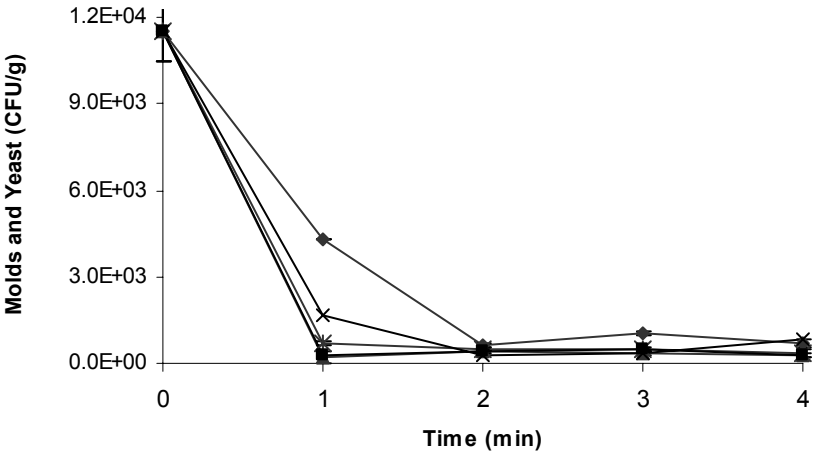
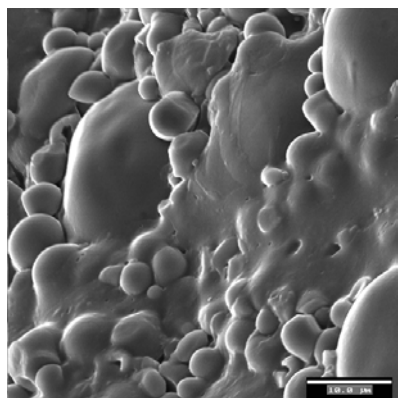
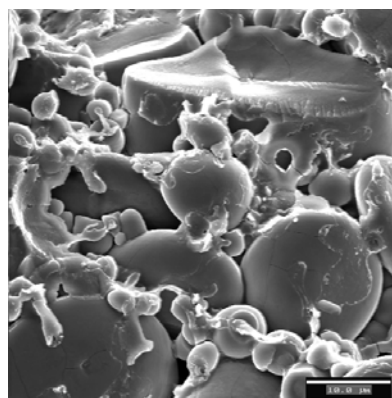


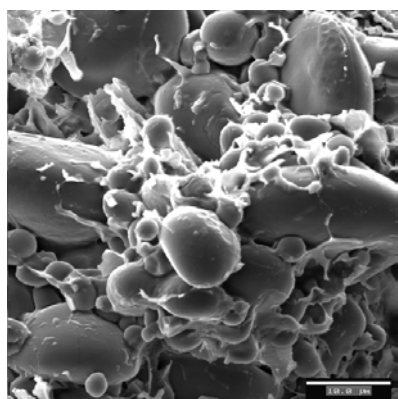
Figure 2



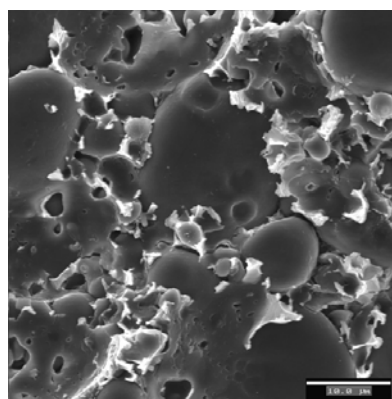
0 MPa



50 MPa

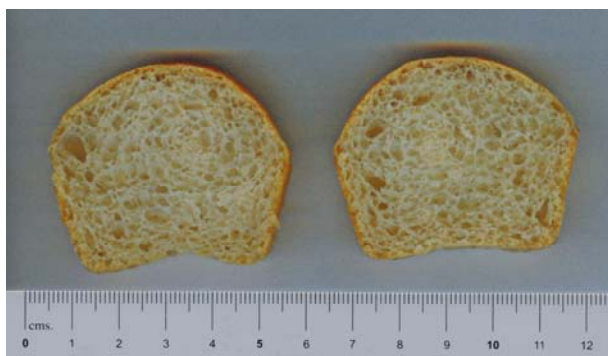


150 MPa



250 MPa

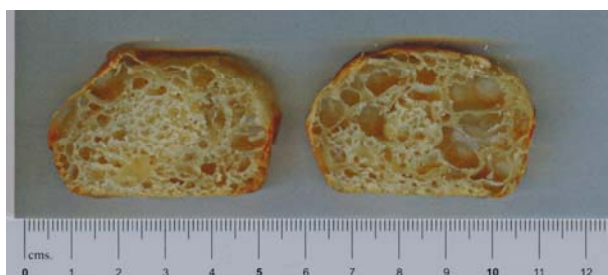
Figure 3.



Control



50 MPa



100 MPa



200 MPa